

Page 10, lines 8-12, insert:

Consensus zinc finger structures may be prepared by comparing the sequences of known zinc fingers, irrespective of whether their binding domain is known. Preferably, the consensus structure is selected from the group consisting of the consensus structure PYKCPECGKSFSQKSDLVKHQRTHTG (SEQ. ID NO.:1), and the consensus structure PYKCSECGKAFSQKSNLTRHQRIHTGEKP (SEQ ID NO.:2).

Page 13, lines 30-33, insert:

Figure 1b shows amino acid sequences of the variant  $\alpha$ -helical regions from some zinc fingers selected by phage display using the DNA binding site gcggnggcg (SEQ. ID NO.:4) where the central (bold) nucleotide of the middle (underlined) triplet was either (I) 5-

Page 14, lines 6-22, insert:

Figure 1c shows a phage ELISA binding assay showing discrimination of pyrimidines by representative phage-selected zinc fingers. The matrix shows three different zinc finger phage clones (x, y and z) reacted with four different DNA binding sites present at a concentration of 3nM. Binding is represented by vertical bars which indicate the OD obtained by ELISA (Choo and Klug, (1997) Curr. Opin. Str. Biol. 7:117-125). The amino acid sequences of the variant  $\alpha$ -helical regions from the selected zinc fingers are: REDVLIRHGK (x) (SEQ. ID NO.: 5), RADALMVHKR (y) (SEQ. ID NO.:6), and RGPDLARHGR (z) (SEQ. ID NO.:7). The DNA sequences contain the generic binding site gcggnggcg (SEQ. ID NO.:4), where the central

Sub 35 cont  
(bold) nucleotide was either : uracil (U), thymine (T), cytosine (C), or 5-methylcytosine (M).

Sub 36  
A5 cont  
Figure 2 shows the effect of cytosine methylation on DNA binding by phage-selected zinc fingers. Graphs show three different zinc finger phage binding to the DNA sequence gcggcggcg (SEQ. ID NO.:4) in the presence (circle) and absence (triangle) of methylation of the central base (bold). The zinc finger clones tested contained variant  $\alpha$ -helical regions of the middle finger as follows: (a) RADALMVHKR (SEQ. ID NO.:6), (b) RGPDLARHGR (SEQ. ID NO.:7) and (c) REDVLIRHGK (SEQ. ID NO.: 5). The respective zinc finger clones preferentially bind their cognate DNA site in the presence, absence, or regardless of cytosine methylation.

Page 17, lines 24-25, insert:

A6  
A "leader" peptide may be added to the N-terminal finger. Preferably, the leader peptide is MAEEKP (SEQ ID NO.:39).

Page 28, lines 21-22, insert:

A7  
5'ctcctgcagt tggacctgtg ccatggccgg ctgggccgca tagaatggaa caactaaagc 3'  
(SEQ ID NO.:11).

Page 32, lines 20-27, insert:

A8  
DNAs of the form 5'-tatagtg-xxxx-ggcgtgtcacagtcagtcacacacgtc-3' (SEQ. ID NO.:12), and their complementary strands, are chemically synthesized and annealed

in 20mM Tris-HCl, pH 8, 100mM NaCl. The DNA sequences -XXXX-represent nucleotide sequences after methylation by *M.HaeIII* (GGMC) or *M.HhaI* (GMGC). Since DNA is chemically synthesized, the DNA sites used in selections incorporate 5-meC (in appropriate positions on both strands) with 100% yield. Selections are also carried out on derivatives of these sites containing thymine rather than 5-meC in the appropriate positions (and with A rather than C on the complementary strand as appropriate).

Page 37, lines 1-10, insert:

apparent  $K_d$  of each clone for the optimally bound DNA site(s) is in the nanomolar range, similar to that of wild-type Zif268 DNA-binding domain for its preferred target site using this assay. The  $K_d$ s obtained are shown in Table 2. Clones zfHAE(M) (Table 1 F1: SEQ ID NO.:20; F2: SEQ ID NO.:25; F3: SEQ ID NO.:30) and zfHHA(M) (Table 1 F1: SEQ ID NO.:21; F2: SEQ ID NO.:26; F3: SEQ ID NO.:31) preferentially bind their respective DNA target sites when 5-meC is incorporated into the correct nucleotide positions, and discriminated against the unmethylated DNA sites by factors of approximately 20-fold and 5-fold, respectively. The discrimination shown by zfHAE(M) in particular is good considering the simple DNA recognition mechanism of zinc fingers, and that only a single functional group per DNA molecule has been altered Clones zfHAE(Y) (Table 1 F1: SEQ ID NO.:23; F2: SEQ ID NO.:27; F3: SEQ ID NO.:32) and zfHHA(Y) (Table 1 F1: SEQ ID NO.:24; F2: SEQ ID NO.:28; F3: SEQ ID NO.:33) bind their respective target sites but do not show any preference for either the modified or unmodified forms.